EVENT ABSTRACT

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Stepwise implementation of high-throughput sequencing metabarcoding to estuarine macrobenthic communities

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Benthic communities are key components of aquatic ecosystems' biomonitoring. However, morphology-based species identifications remain a low-throughput, and sometimes ambiguous, approach. Despite metabarcoding methodologies have been applied for above-species taxa inventories in marine meiofaunal communities, a comprehensive approach providing species-level identifications for estuarine macrobenthic communities is still lacking. Here we report a combination of experimental and field studies that demonstrate the aptitude of cytochrome oxidase I (COI) metabarcoding to provide robust species-level identifications within a framework of high-throughput monitoring of estuarine macrobenthic communities.

This study was designed in two main sequential phases. The first phase focused on analysis of macrobenthic communities with known composition, while the second phase comprised natural field-collected macrobenthic communities. To investigate the ability to recover DNA barcodes from all species present in a bulk community DNA extract, we assembled experimentally 3 phylogenetically diverse communities comprising mollusks, crustaceans and annelids species, therefore representing the 3 most dominant taxa in typical estuarine macrobenthic communities. Four different primer pairs were used to generate an equal number of different PCR products of the COI barcode region. In the second phase, we compared morphology and metabarcoding-based approaches (using the most successful primer sets tested in the first phase) to determine the species composition of macrobenthos from four distinct sites of the Sado estuary, Portugal. At each site, organic matter content (extrapolated from total combustible carbon, TOM) and fine fraction (particle size < 63 μm) were determined.

In the first phase, between 78 and 83% of the species in the tested communities were recovered through primer-multiplexed high throughput sequencing (HTS). Two primer pairs were sufficient to attain these recovery rates. In the second phase, the sediments' types in the 4 sites of the Sado estuary sampled for the natural macrobenthic communities varied considerably in their features, ranging from sandy to muddy sediments. Globally, 55 taxa (five phyla) were morphologically identified in the natural communities, 27 of which were identified to species level (four phyla) and the remaining 28 to higher taxonomic ranks. Metabarcoding-based identification generated a total of 61 species (six phyla) matches in all 4 natural communities, obtained through searches against both our own reference library and GenBank public database.

Our study demonstrates the aptitude of COI metabarcoding using HTS approach for implementation in biodiversity assessments of estuarine macrobenthic communities. High-throughput metabarcoding may enable more frequent and spatially detailed biomonitoring with higher information content, concomitantly reducing time and cost constraints in the monitoring of benthic communities. By virtue of the generation of readily comparable DNA sequence data, the metabarcoding approach can provide species-level information of high quality, with reduced ambiguity and susceptible to scrutiny in the future. The ability to provide data on parasite occurrence, for example, and to enable early detection of alien species, or to discriminate cryptic species, constitute highly relevant additional benefits of this approach. Nevertheless, further refinement is still required, to improve its overall efficiency and output, namely the improvement of the recovery rates through the refinement of primers and testing of alternative combinations, especially for the recalcitrant species. Lastly, the continuing completion of the still incipient reference libraries of DNA barcodes for marine invertebrates will be decisive to fully materialize the potential of metabarcoding.

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